



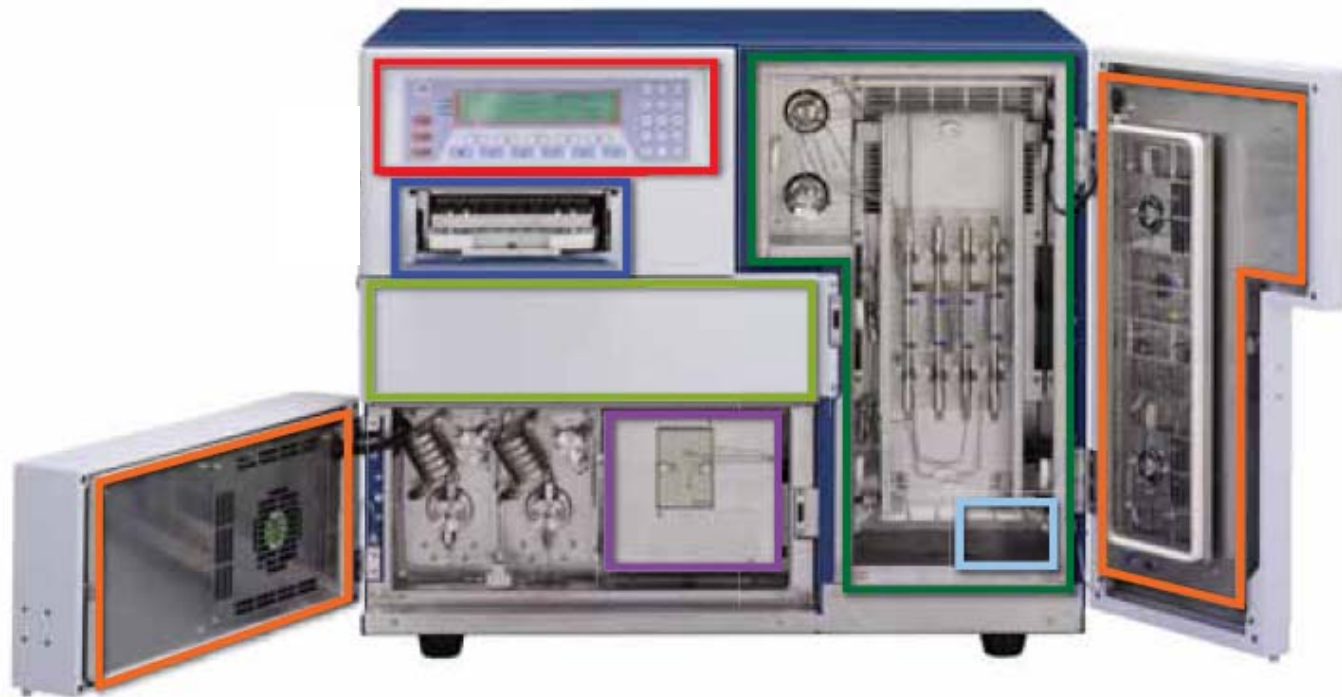
# **Effect of Temperature and Flow Rate Changes on Molecular Weight Accuracy and Precision of the EcoSEC<sup>®</sup> GPC System**

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Technical Service Specialist  
Tosoh Bioscience LLC



# EcoSEC GPC System

*All-In-One-System*

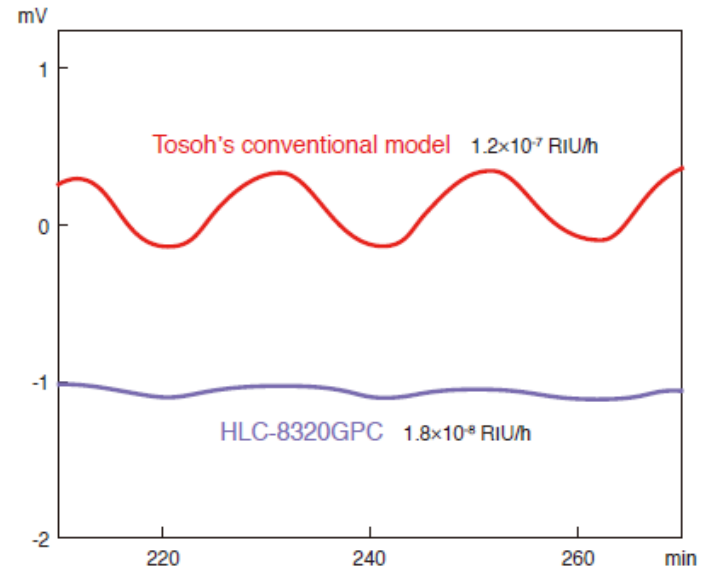




# High Stability – Column Oven



Baseline fluctuation due to RT variation

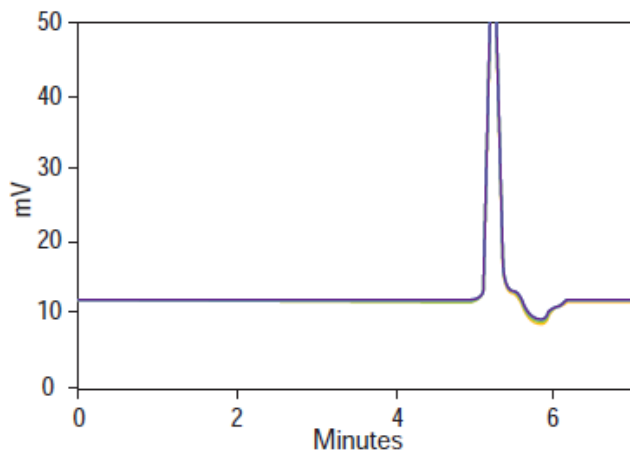


Column: TSKgel SuperMultiporeHZ-M × 2  
Eluent: Chloroform  
Flow rate: 0.35 mL/min  
Temperature: 23 to 28 °C (Swing: 5°C/10 min). Measured in the environmental testing room.  
Detection: RI



# High Stability – Dual Flow RI Detector

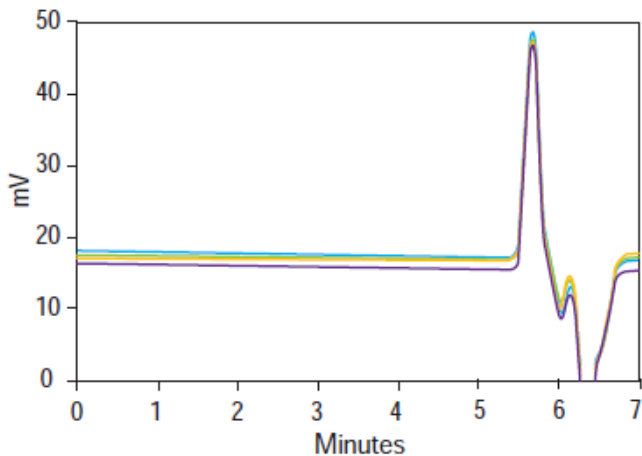
## EcoSEC with dual-flow RI



TSKgel SuperMultiporeHZ-M,  
4.6mm ID x 15cm

Mobile Phase: THF  
Flow rate: 0.35mL/min  
Detection: RI  
Temperature: 40°C  
Injection vol.: 10µL  
Sample: dicyclohexyl phthalate

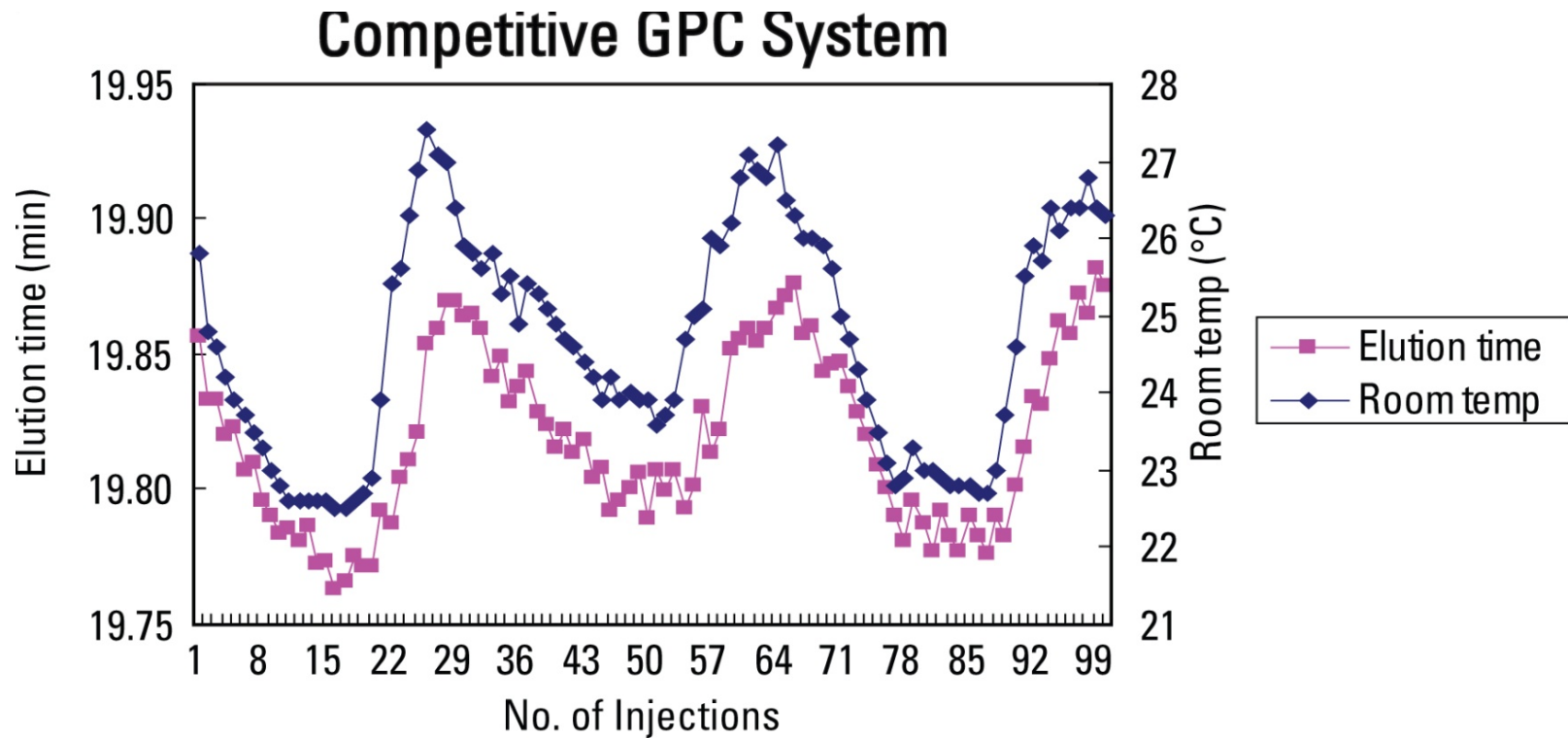
## GPC with single-flow RI





# High Reproducibility – Pump Oven

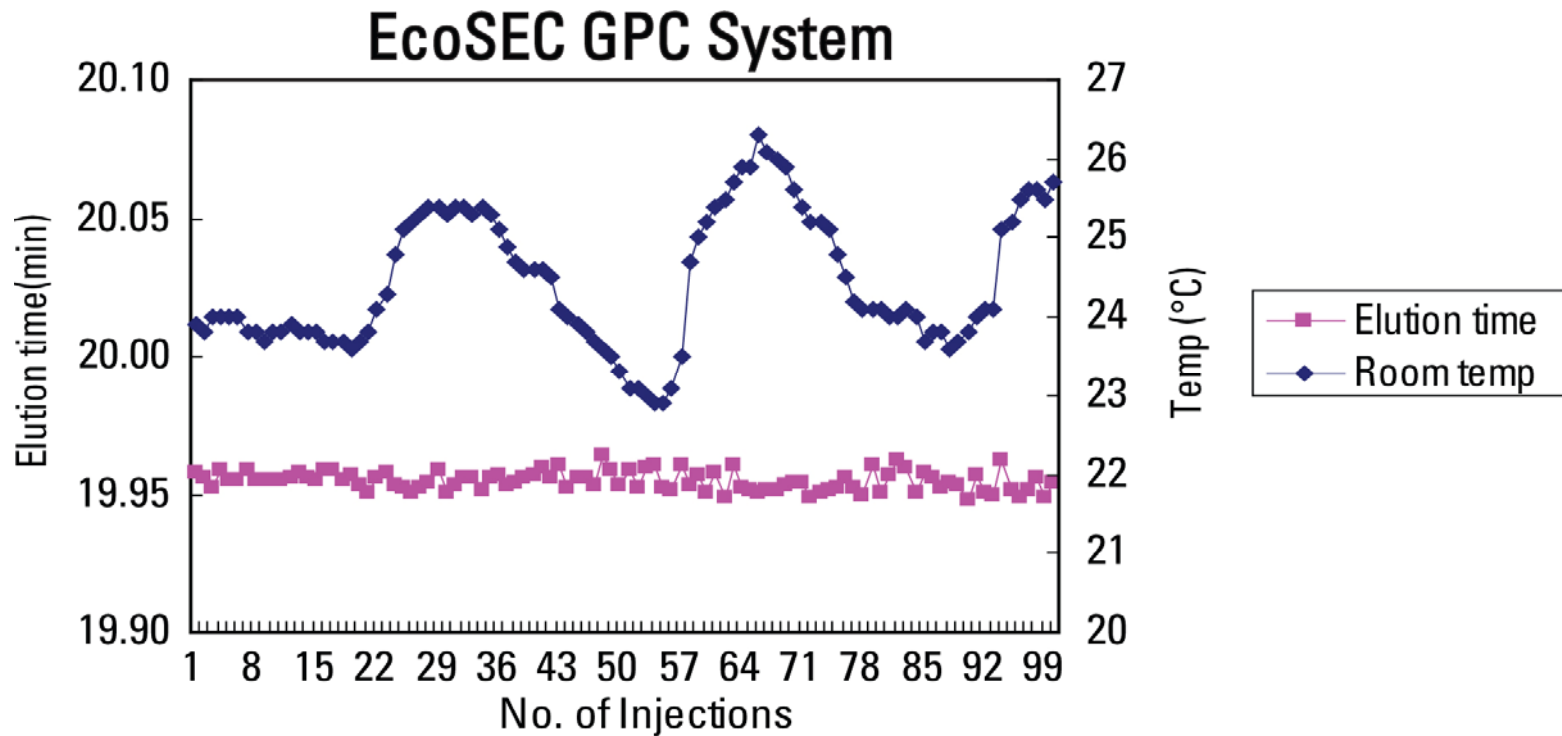
- Room temperature affects retention time
  1. Conventional instrument - Pump *not* temperature controlled





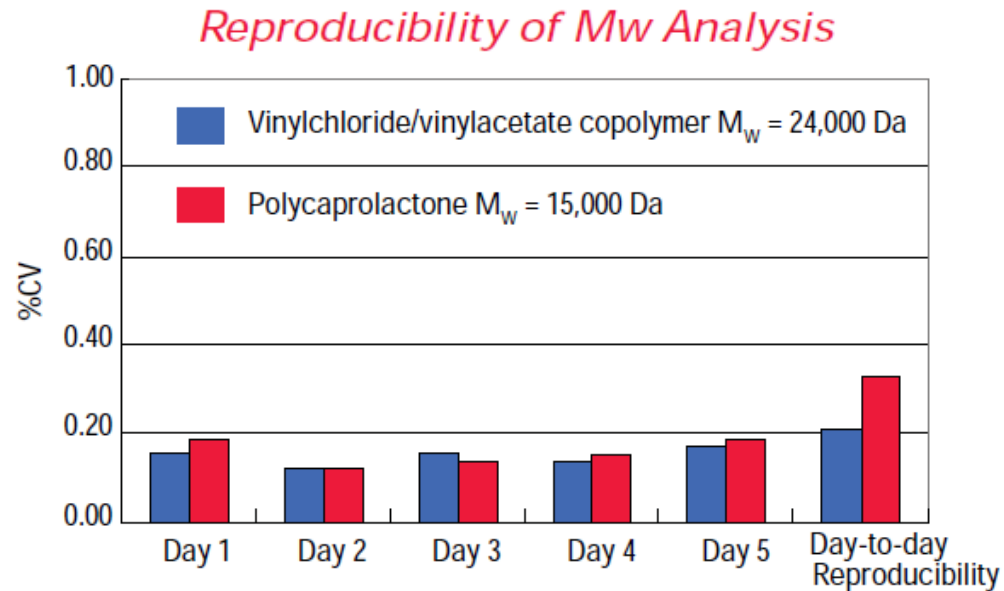
# High Reproducibility – Pump Oven

- Room temperature affects retention time
- 2. EcoSEC GPC System – Pumps are temperature controlled





# High Reproducibility – MW Analysis



CV value less than 0.2% or less a day.

CV value less than 0.4% on different days.

**TSKgel SuperMultiporeHZ-M, 4.6mm ID x 15cm, × 2**

Mobile phase: THF

Flow rate: 0.35mL/min

Temperature: 40°C

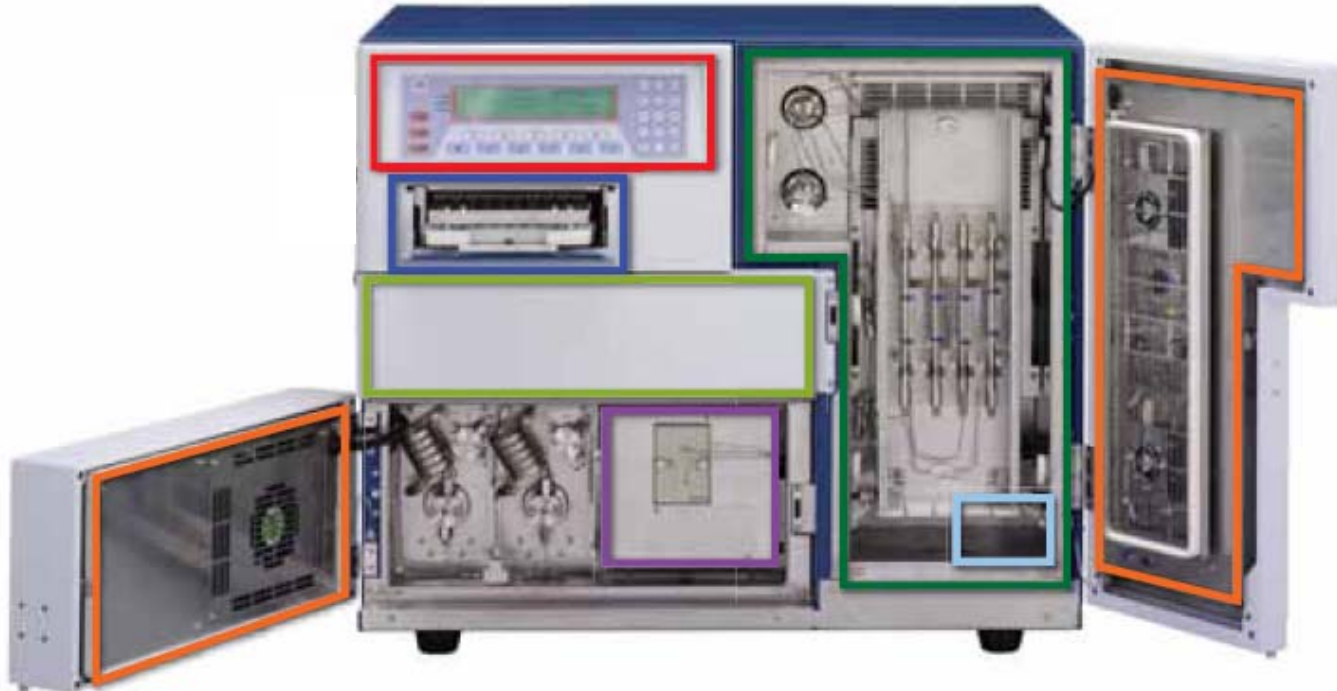
Injection vol.: 10µL

Samples: copolymer and polyester standards



## High Performance – Low Dead Volume System

- Low dead volume design and system layout
  - 7.5 $\mu$ L Pump stroke volume
  - 2.5 $\mu$ L RI cell volume
  - Use of 0.2mm and 0.4mm ID tubing



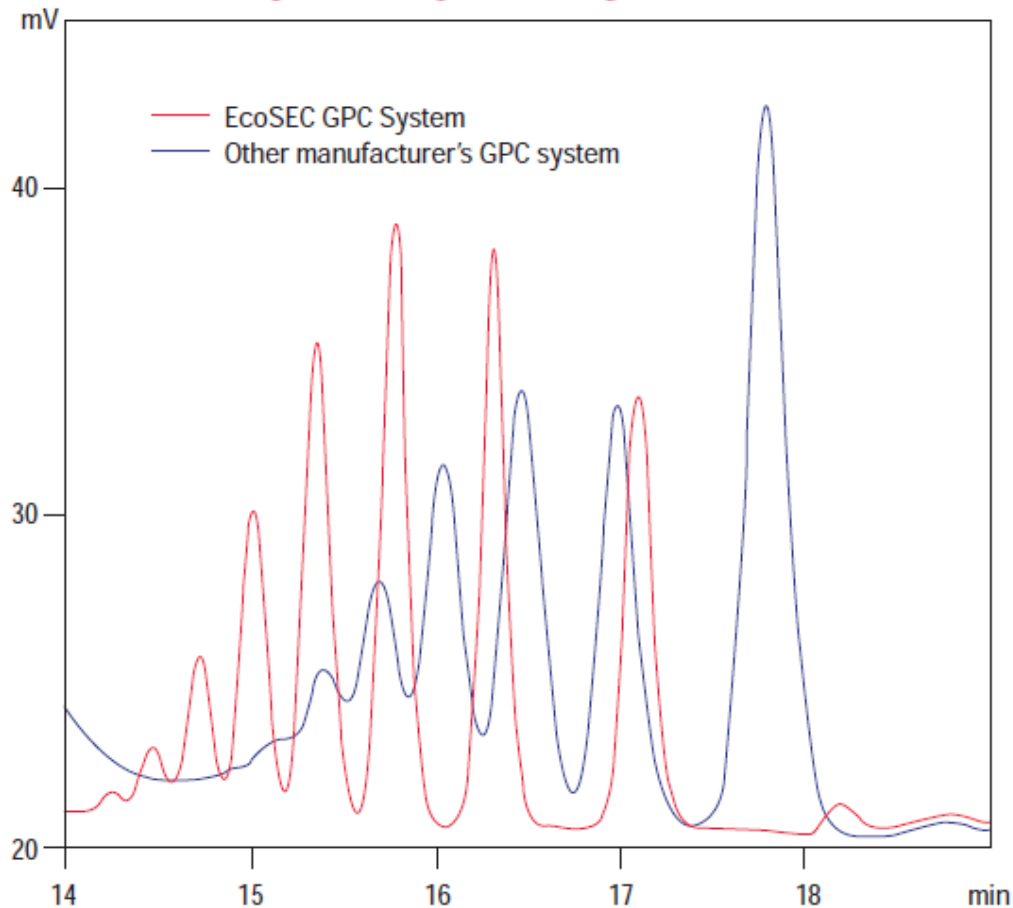




# High Performance – High Resolution

- Low dead volume system offers better resolution for semi-micro columns

## Analysis of Styrene Oligomer (A-500)



TSKgel SuperHZ2000, 4.6mm ID x 15cm, x 4

Mobile phase: THF

Flow rate: 0.35mL/min

Detection: RI

Temperature: 40°C

Injection vol.: 10 $\mu$ L (0.2mg/mL)

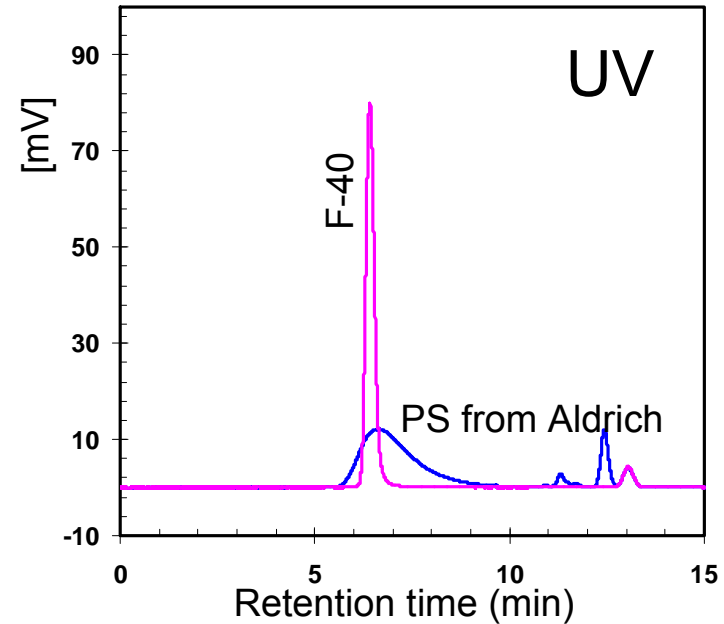
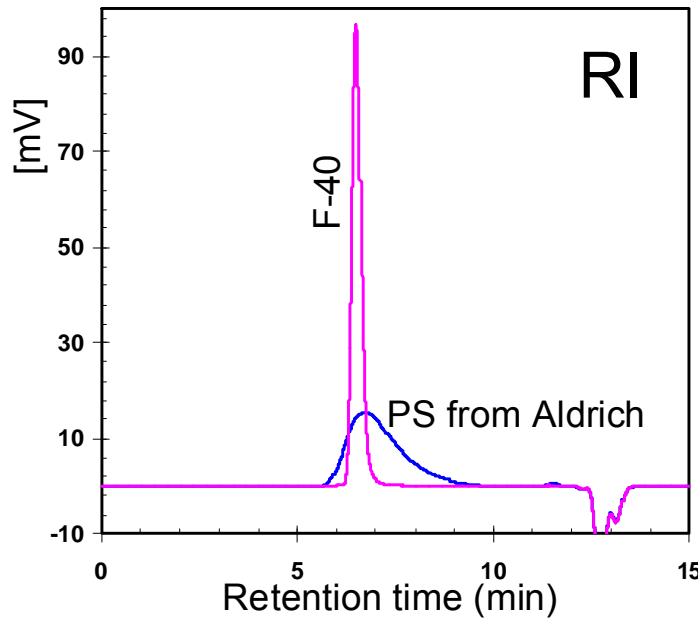


# Methods

- Two kinds of polystyrene samples:
  - monodisperse
    - F-40: Mw  $4.27 \times 10^5$  Da, PDI 1.02;
  - polydisperse
    - PS from Aldrich: Mw  $2.9 \times 10^5$  Da, PDI 2.23;
- Temperature range: 40 °C
  - 30 - 50 °C;
- Flow rate range: 0.35 mL/min
  - 0.30 mL/min – 0.40 mL/min;
- Columns: TSK-GEL SuperMultiporeHZ-M (x2)
- Eluent: THF
- Detection: RI & UV
- Injection volume: 10  $\mu$ L



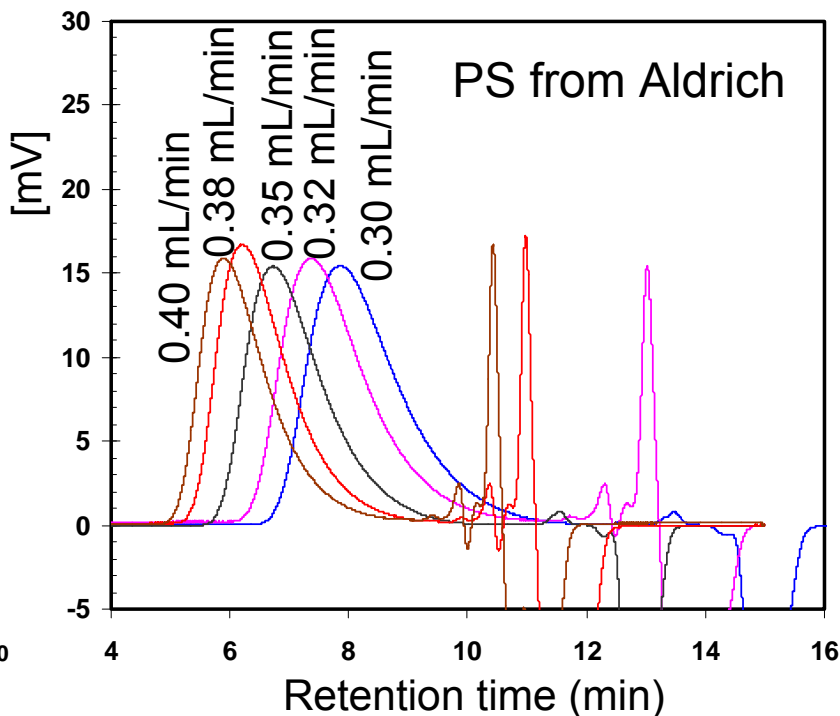
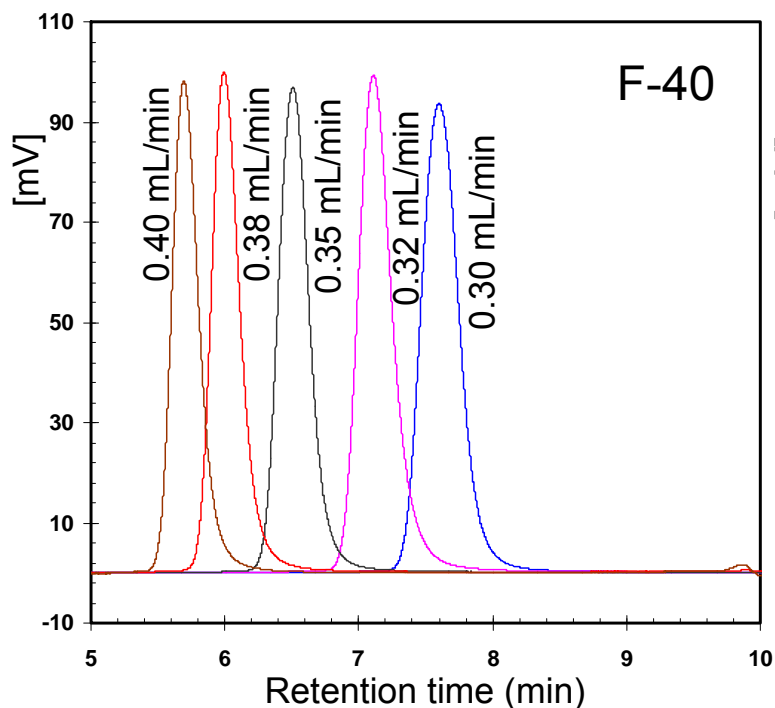
# Polystyrene samples



Column: TSKgel SuperMultiporeHZ-M (x2) (4.6mm I.D. x 15cm)  
Mobile Phase: THF  
Flow rate: **0.35 mL/min**  
Detection: RI and UV  
Temperature: **40°C**  
Injection vol.: 10 $\mu$ L



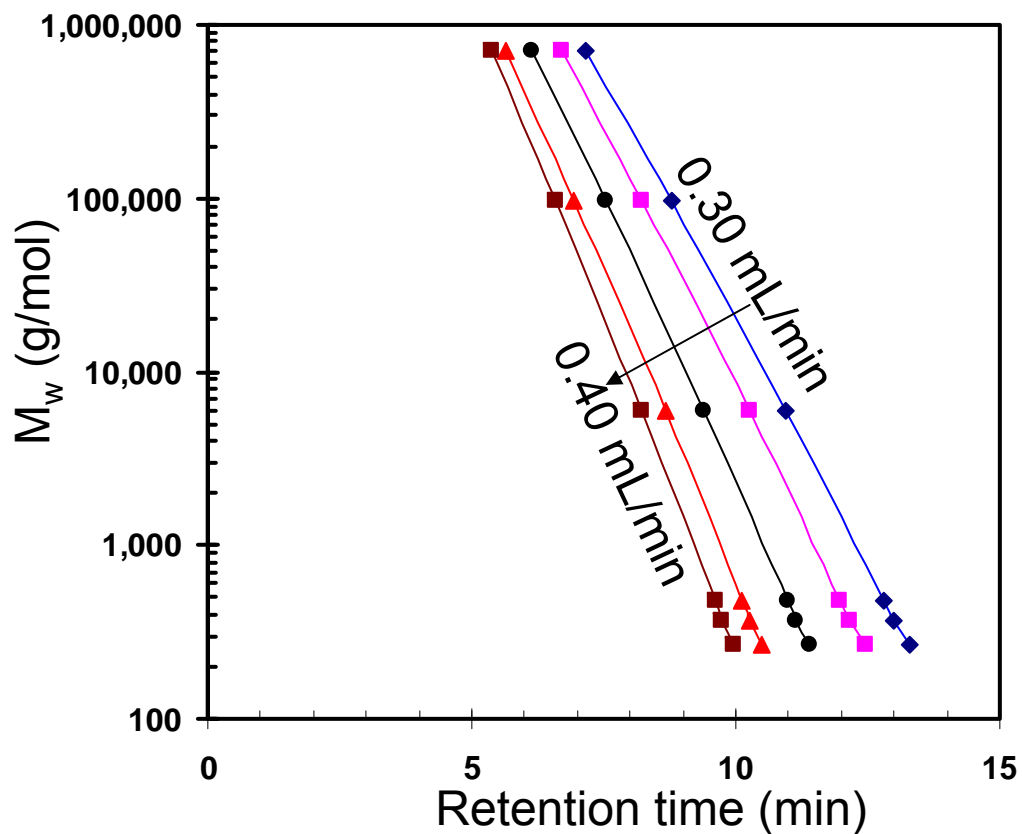
# Flow rate dependence



Column: TSKgel SuperMultiporeHZ-M (x2) (4.6mm I.D. x 15cm)  
Mobile Phase: THF  
**Flow rate: 0.30 – 0.40 mL/min**  
Detection: RI  
Temperature: 40°C  
Injection vol.: 10µL



# Calibration curve vs. flow rate



Calibration curves are based on PStQuickMP-M kit



# MW/MWD determination vs. flow rate

Retention time and calculated MW/MWD of F-40 with different flow rates at 40 °C

F-40 PS flow rates	RI detection					UV detection				
	Ret. Time	Mw		Mw/Mn		Ret. Time	Mw		Mw/Mn	
0.30mL/min	7.600	85,323	-79%	1.05	1%	7.478	88,637	-78%	1.04	1%
0.31mL/min	7.343	121,770	-70%	1.05	1%	7.227	125,332	-69%	1.04	1%
0.32mL/min	7.109	170,526	-58%	1.05	1%	7.001	173,730	-57%	1.04	1%
0.33mL/min	6.896	231,879	-43%	1.04	0%	6.795	233,463	-43%	1.04	1%
0.34mL/min	6.702	408,249	0%	1.03	0%	6.601	409,594	1%	1.03	0%
<b>0.35mL/min</b>	<b>6.507</b>	<b>406,559</b>		<b>1.03</b>		<b>6.410</b>	<b>406,866</b>		<b>1.03</b>	
0.36mL/min	6.325	515,223	27%	1.03	0%	6.232	512,017	26%	1.03	0%
0.37mL/min	6.165	641,423	58%	1.03	0%	6.073	638,075	57%	1.03	0%
0.38mL/min	5.993	808,849	99%	1.03	-1%	5.901	801,948	97%	1.03	0%
0.39mL/min	5.854	978,364	141%	1.03	-1%	5.764	965,943	137%	1.03	0%
0.40mL/min	5.693	1,209,458	197%	1.02	-1%	5.608	1,189,039	192%	1.02	-1%

F-40: Mw  $4.27 \times 10^5$  Da, PDI 1.02 (manufacturer's data)

MW/MWD calculated based on 0.35 mL/min calibration curve



# MW/MWD determination vs. flow rate

Retention time and calculated MW/MWD of PS from Aldrich with different flow rates at 40 °C

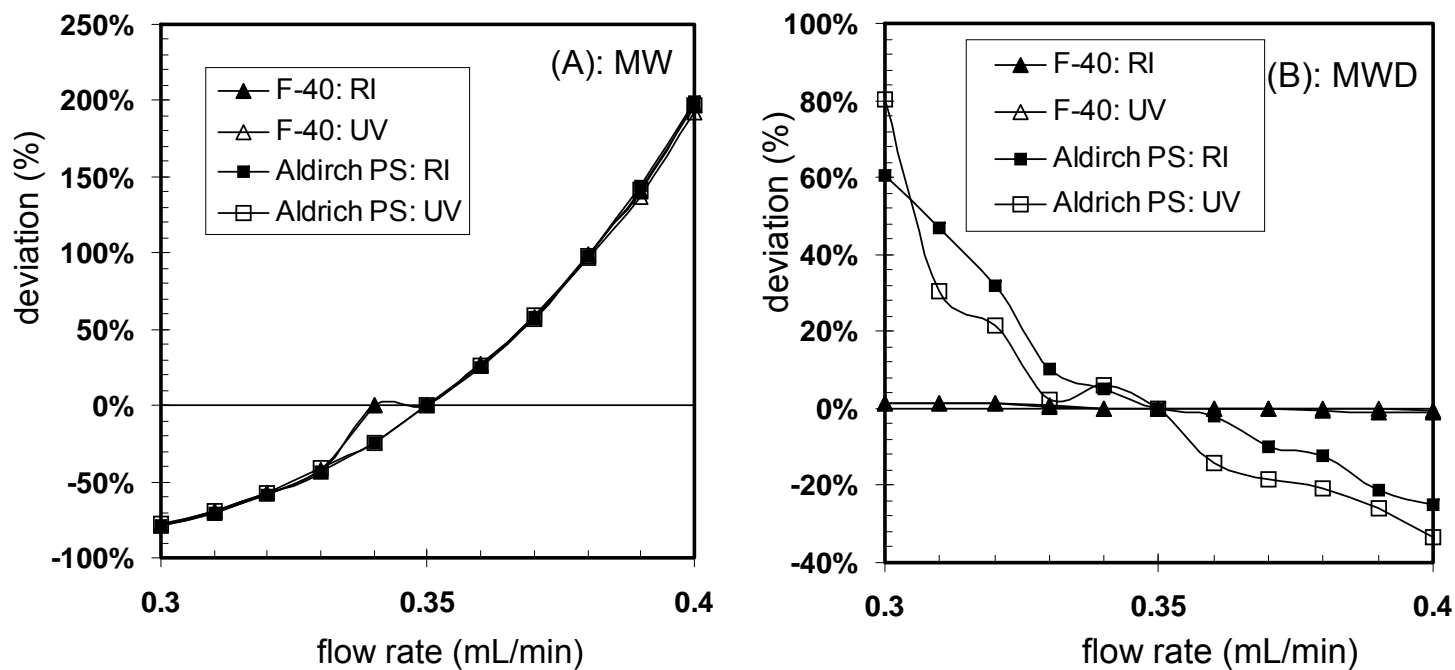
Aldrich PS flow rates	RI detection					UV detection				
	Ret. Time	Mw	Mw/Mn			Ret. Time	Mw	Mw/Mn		
0.30mL/min	7.849	55,684	-79%	4.88	61%	7.720	57,236	-78%	6.58	80%
0.31mL/min	7.619	77,767	-71%	4.46	47%	7.485	79,594	-70%	4.75	30%
0.32mL/min	7.361	110,347	-58%	4.01	32%	7.248	111,394	-57%	4.44	22%
0.33mL/min	7.138	151,569	-43%	3.35	10%	7.037	153,170	-41%	3.72	2%
0.34mL/min	6.935	200,375	-25%	3.20	5%	6.829	198,638	-24%	3.86	6%
<b>0.35mL/min</b>	<b>6.723</b>	<b>265,657</b>		<b>3.04</b>		<b>6.618</b>	<b>261,552</b>		<b>3.65</b>	
0.36mL/min	6.551	331,815	25%	2.99	-2%	6.453	328,404	26%	3.14	-14%
0.37mL/min	6.378	420,110	58%	2.73	-10%	6.283	414,768	59%	2.97	-18%
0.38mL/min	6.209	526,065	98%	2.67	-12%	6.113	518,321	98%	2.89	-21%
0.39mL/min	6.056	646,914	144%	2.39	-21%	5.961	633,228	142%	2.70	-26%
0.40mL/min	5.894	796,428	200%	2.29	-25%	5.806	777,287	197%	2.43	-33%

PS from Aldrich: Mw  $2.9 \times 10^5$  Da, PDI 2.23 (manufacturer's data)

MW/MWD calculated based on 0.35 mL/min calibration curve



# MW/MWD deviation

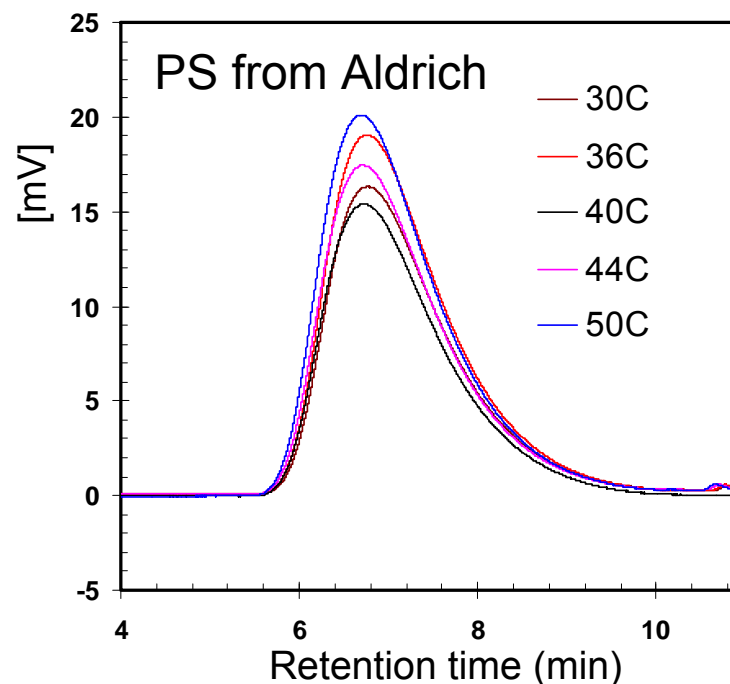
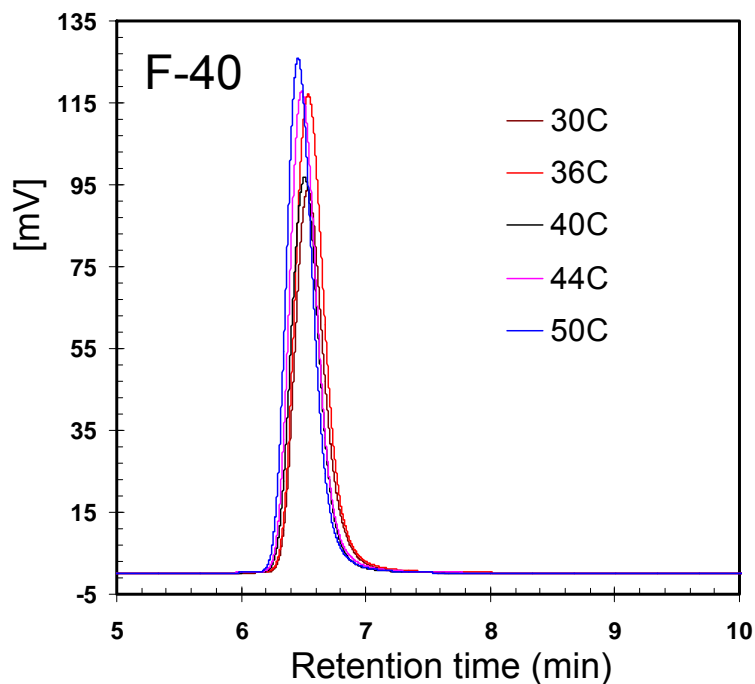


Reference flow rate is 0.35 mL/min





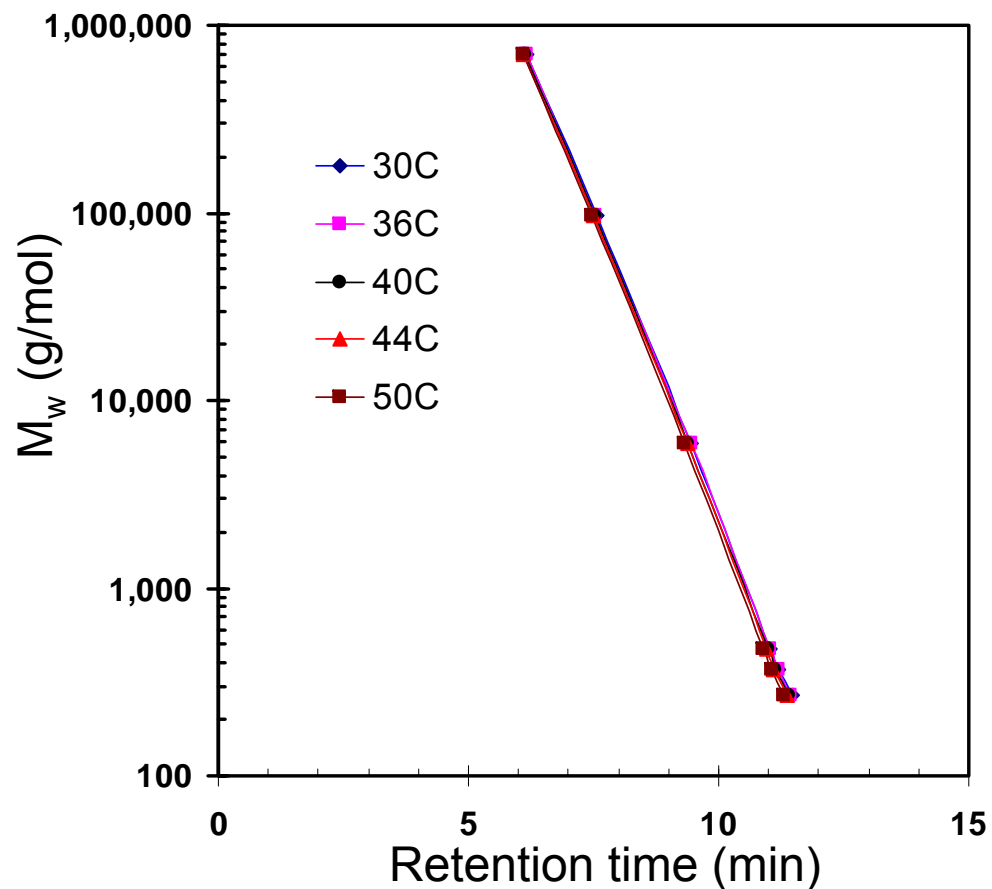
# Temperature dependence



Column: TSKgel SuperMultiporeHZ-M (x2) (4.6mm I.D. x 15cm)  
Mobile Phase: THF  
Flow rate: 0.35 mL/min  
Detection: RI  
**Temperature: 30 - 50°C**  
Injection vol.: 10 $\mu$ L



# Calibration curve



thermodynamic equilibrium

$$\Delta G^0 = -RT \ln K$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0$$

$$\ln K_{SEC} = \frac{\Delta S^0}{R}$$

Calibration curves are based on PStQuickMP-M kit



# MW/MWD determination vs. temperature

Retention time and calculated MW/MWD of F-40 with different temperature at 0.35 mL/min

F-40 PS Temperature	RI detection					UV detection				
	Ret. Time	Mw	Mw/Mn			Ret. Time	Mw	Mw/Mn		
30°C	6.535	383374	-6%	1.04	1%	6.443	381046	-7%	1.04	1%
32°C	6.536	383127	-6%	1.04	1%	6.444	381976	-6%	1.04	1%
34°C	6.533	385919	-6%	1.04	1%	6.440	383805	-6%	1.04	1%
36°C	6.534	385396	-6%	1.04	1%	6.440	384100	-6%	1.03	0%
38°C	6.519	393968	-4%	1.03	0%	6.426	389983	-4%	1.03	0%
<b>40°C</b>	<b>6.507</b>	<b>408599</b>		<b>1.03</b>	0%	<b>6.410</b>	<b>407542</b>		<b>1.03</b>	0%
42°C	6.484	412896	1%	1.03	0%	6.391	410338	1%	1.03	0%
44°C	6.484	412640	1%	1.03	0%	6.390	411073	1%	1.03	0%
46°C	6.469	420727	3%	1.03	0%	6.376	419392	3%	1.03	0%
48°C	6.475	426862	4%	1.03	0%	6.366	424463	4%	1.03	0%
50°C	6.458	429063	5%	1.03	0%	6.364	426242	5%	1.03	0%

F-40: Mw 4.27x10<sup>5</sup> Da, PDI 1.02 (manufacturer's data)

MW/MWD calculated based on 40°C calibration curve



# MW/MWD determination vs. temperature

Retention time and calculated MW/MWD of PS from Aldrich with different temperature at 0.35 mL/min

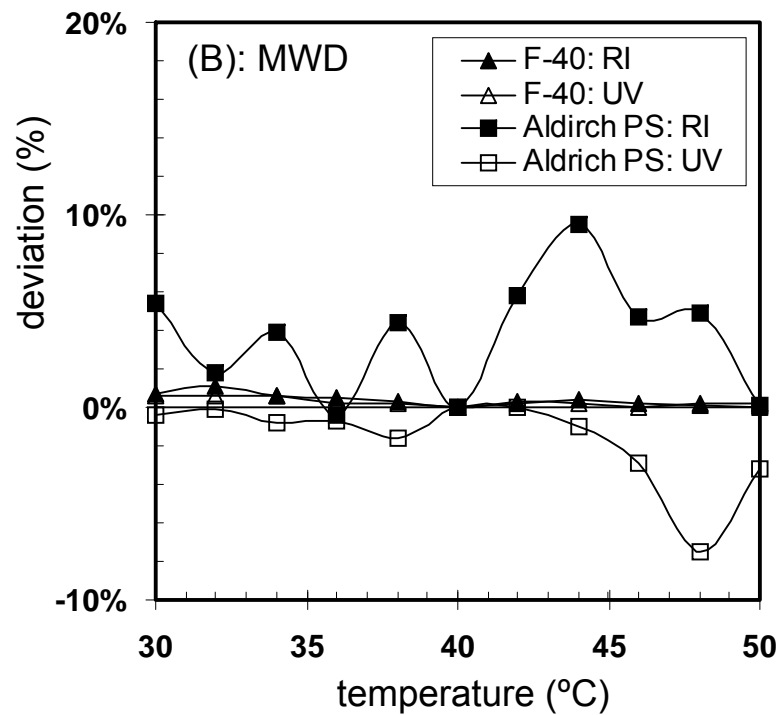
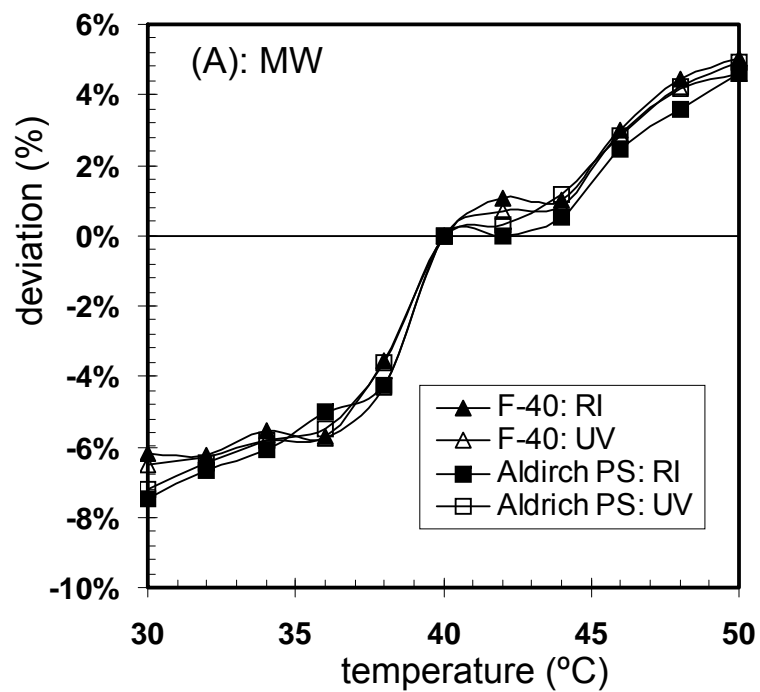
Aldrich PS Temperature	RI detection					UV detection				
	Ret. Time	Mw	Mw/Mn			Ret. Time	Mw	Mw/Mn		
30°C	6.764	245751	-7%	3.20	5%	6.668	242667	-7%	3.63	0%
32°C	6.762	247987	-7%	3.09	2%	6.669	244615	-6%	3.64	0%
34°C	6.764	249550	-6%	3.16	4%	6.659	246381	-6%	3.62	-1%
36°C	6.760	252355	-5%	3.03	0%	6.660	247214	-5%	3.62	-1%
38°C	6.751	254347	-4%	3.17	4%	6.653	252085	-4%	3.59	-2%
<b>40°C</b>	<b>6.723</b>	<b>265657</b>		<b>3.04</b>		<b>6.618</b>	<b>261552</b>		<b>3.65</b>	
42°C	6.716	265551	0%	3.21	6%	6.613	262344	0%	3.65	0%
44°C	6.716	267071	1%	3.33	10%	6.616	264551	1%	3.61	-1%
46°C	6.702	272238	2%	3.18	5%	6.596	268897	3%	3.54	-3%
48°C	6.695	275183	4%	3.19	5%	6.605	272594	4%	3.38	-7%
50°C	6.692	277891	5%	3.04	0%	6.594	274475	5%	3.53	-3%

PS from Aldrich: Mw  $2.9 \times 10^5$  Da, PDI 2.23 (manufacturer's data)

MW/MWD calculated based on 40°C calibration curve

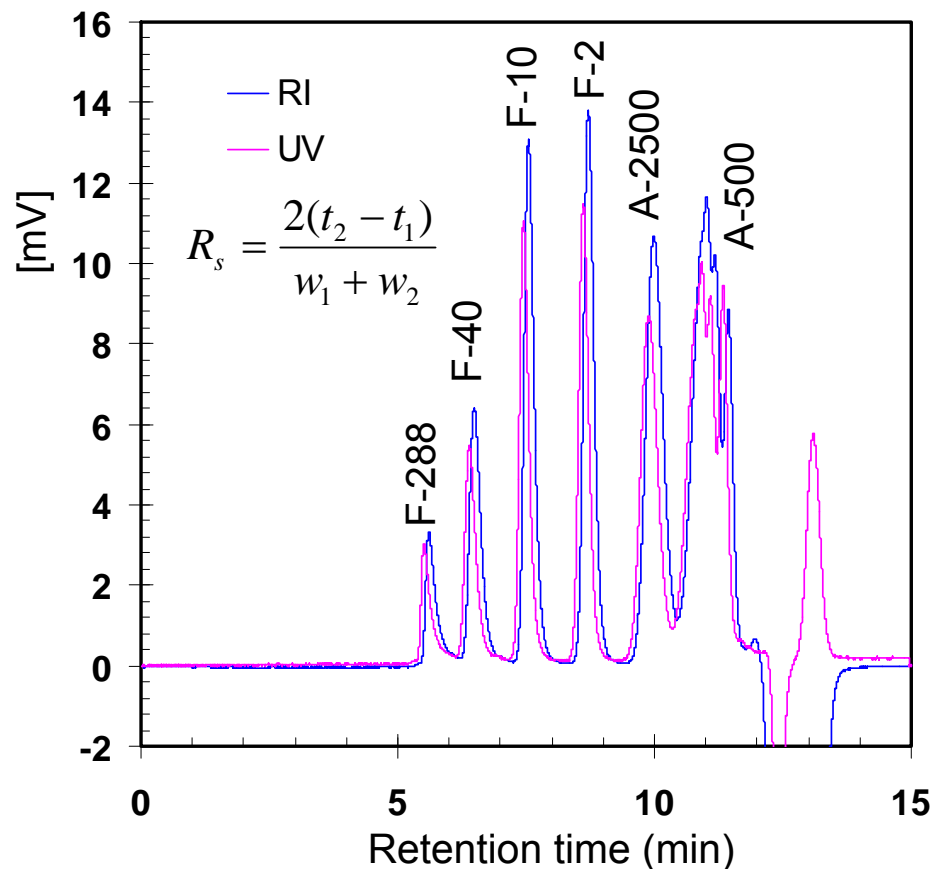


# MW/MWD deviation





# Separation resolution

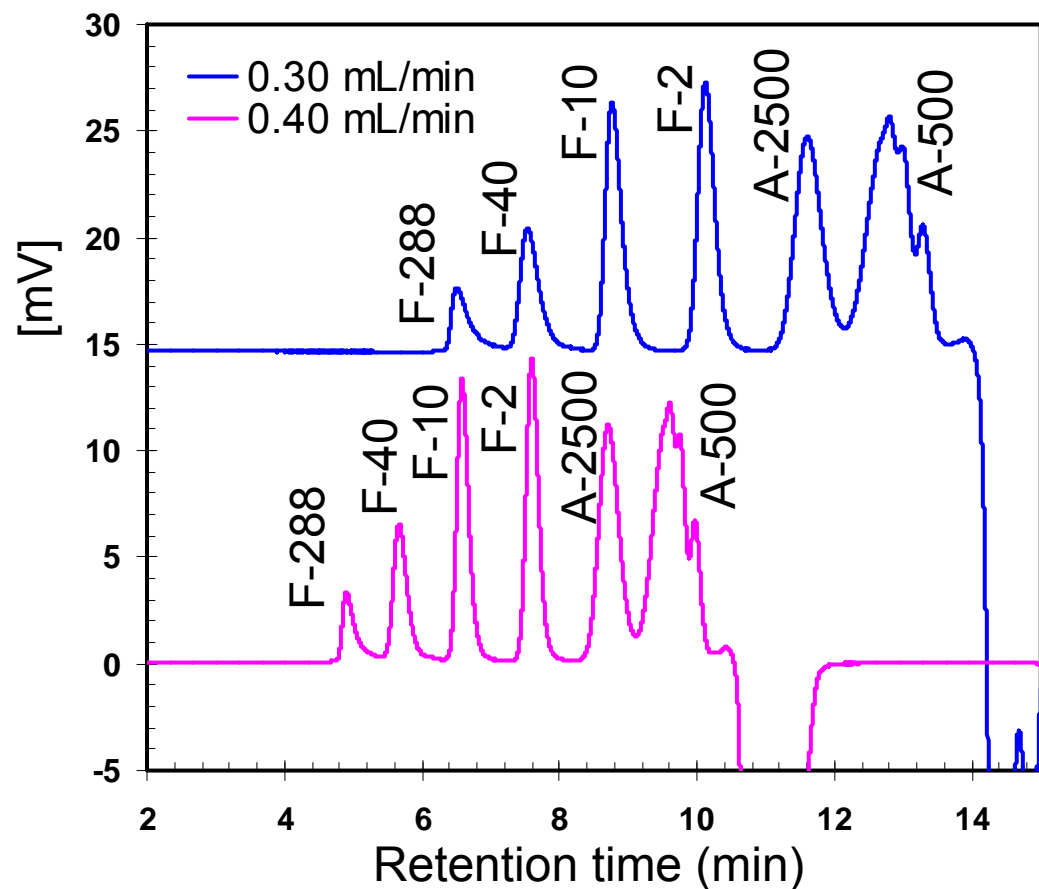


Mixture of PS	Dalton
F-288	$2.89 \times 10^6$
F-40	$4.27 \times 10^5$
F-10	$9.64 \times 10^4$
A-2500	$2.63 \times 10^3$
A-500	500

Column: TSKgel SuperMultiporeHZ-M (x2) (4.6mm I.D. x 15cm)  
 Mobile Phase: THF  
 Flow rate: 0.35 mL/min  
 Detection: RI and UV  
 Temperature: 40°C  
 Injection vol.: 10 µL



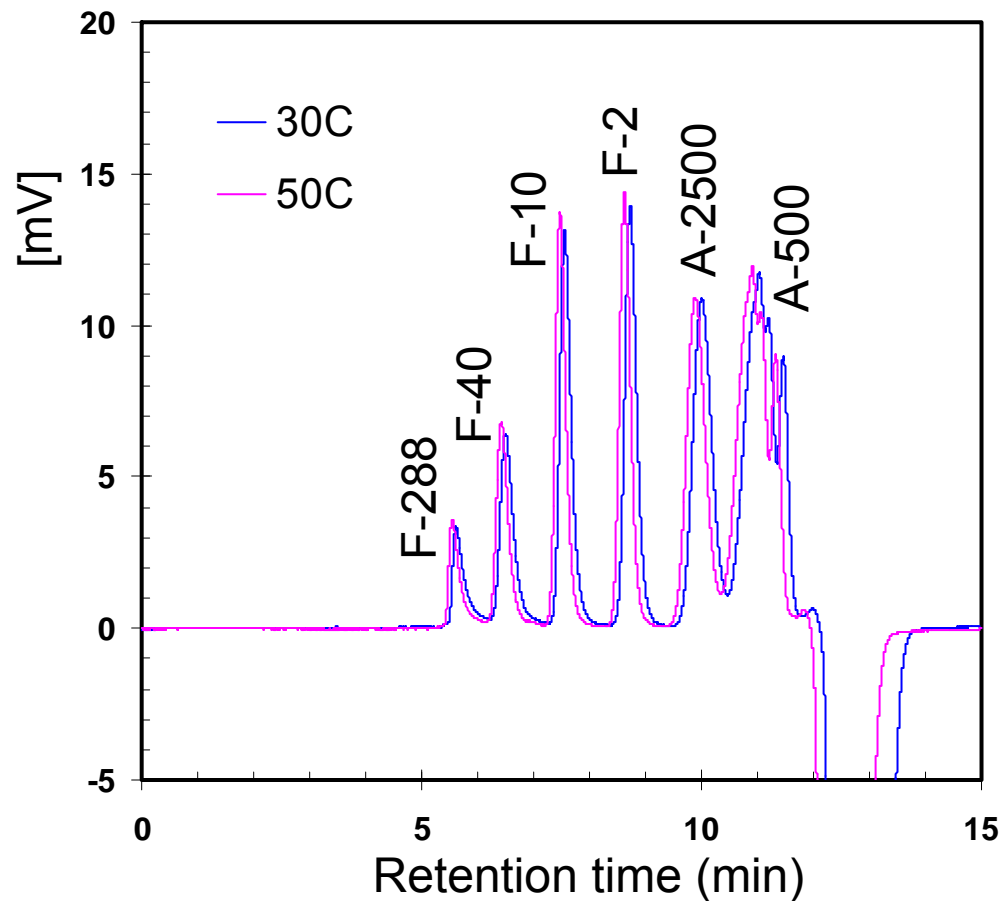
# Resolution (Rs) vs. flow rate



Rs of F-40&F-10		
Flow rates	Rs(UV)	Rs (RI)
0.30mL/min	2.90	2.62
0.31mL/min	2.87	2.63
0.32mL/min	2.86	2.64
0.33mL/min	2.86	2.68
0.34mL/min	2.85	2.68
0.35mL/min	2.86	2.71
0.36mL/min	2.86	2.69
0.37mL/min	2.85	2.65
0.38mL/min	2.83	2.62
0.39mL/min	2.83	2.62
0.40mL/min	2.83	2.62



# Resolution vs. temperature

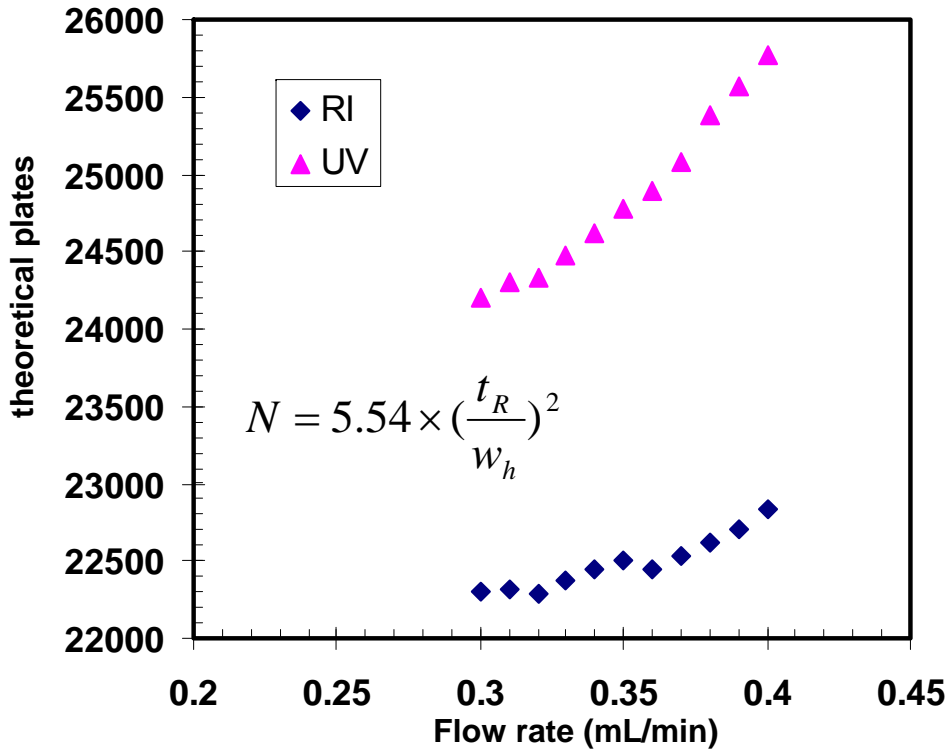


Rs of F-40&F-10		
Temperature	Rs(UV)	Rs (RI)
30°C	2.78	2.63
32°C	2.80	2.65
34°C	2.81	2.66
36°C	2.81	2.67
38°C	2.85	2.69
<b>40°C</b>	<b>2.86</b>	<b>2.71</b>
42°C	2.88	2.73
44°C	2.91	2.75
46°C	2.94	2.78
48°C	2.96	2.80
50°C	2.98	2.81



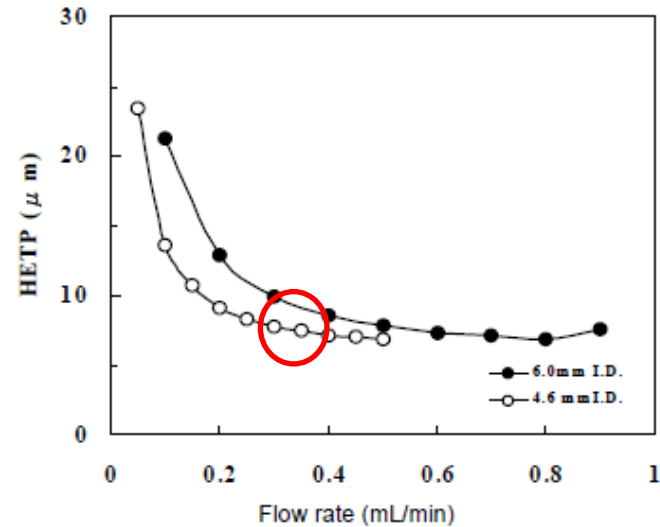


# Column efficiency vs. flow rate



DCHP: dicyclohexyl phthalate

Flow rate dependency of HETP of DCHP on TSKgel SuperMultiporeHZ-M



Column size: 6mm I.D. x 15 cm,  
4.6 mm I.D. x 15 cm

Eluent: THF

Temperature: Ambient

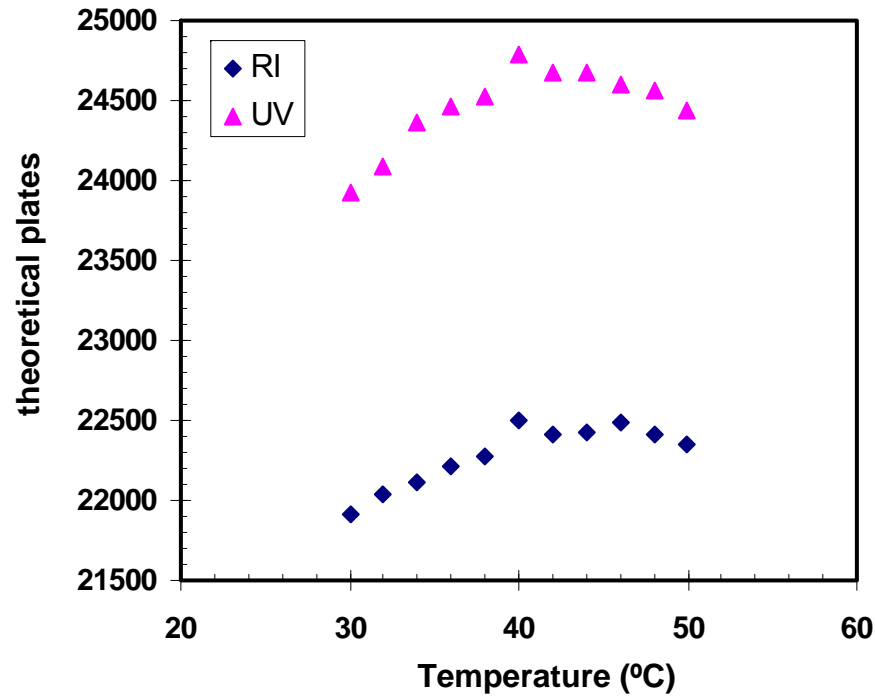
Sample: DCHP

Injection volume: 2uL for 6mm I.D. column,  
1uL for 4.6mm I.D. column

Detector: UV (254 nm)



# Column efficiency vs. temperature





# Summary and conclusions (MW/MWD)

Reference flow rate: **0.35 mL/min**; reference temperature: **40 °C**  
 Flow rate range: 0.35±0.05 mL/min, temperature range: 40±10 °C

Significant	Lower flow rate	Higher flow rate
Monodisperse PS	Underestimate MW No effect on MWD	Overestimate MW No effect on MWD
Polydisperse PS	Underestimate MW Overestimate MWD	Overestimate MW Underestimate MWD
Minor	Lower T	Higher T
Monodisperse PS	Underestimate MW No effect on MWD	Overestimate MW No effect on MWD
Polydisperse PS	Underestimate MW Fluctuation on MWD	Overestimate MW Fluctuation on MWD



# Summary and conclusions (Rs & N)

Reference flow rate: **0.35 mL/min**; reference temperature: **40 °C**  
Flow rate range:  $0.35 \pm 0.05$  mL/min, temperature range:  $40 \pm 10$  °C

<b>Minor</b>	<b>Lower flow rate</b>	<b>Higher flow rate</b>
Resolution	Negligible	Negligible
Column efficiency*	Lower efficiency	Higher efficiency
<b>Obvious</b>	<b>Lower temperature</b>	<b>Higher temperature</b>
Resolution	Lower resolution	Higher resolution
Column efficiency	Lower efficiency	Higher efficiency up to a plateau

\* Below the optimal flow rate.



# Thank you for your attention!

